

b'
wcl.

targeted protein and the assay plate is often quite uneven. This renders the streamlining of the assay in a high throughput format almost impossible to achieve.

At page 4, sixth paragraph:

14) A recombinant polypeptide according to 13), having at least 80% amino-acid identity with a polypeptide comprising:

B²
Sub D7

- from amino acid 1 to between amino acids 1027 and 1062 of the amino acid sequence of SEQ ID N°20, or
- from amino acid 1 to between amino acids 1019 and 1079 of the amino acid sequence of SEQ ID N°22

At page 9, first paragraph:

B³
Sub D2

their native folding and hence their [³H]gabapentin binding properties are those corresponding to the native protein in which amino-acid stretch __984__ to __C-terminal end__ of the amino-acid sequence of SEQ ID N°22 has been deleted. The skilled scientist can quite easily determine within this amino-acid stretch the optimal $\alpha_2\delta$ -3 subunit polypeptides.

At page 10, second full paragraph:

B⁴
Sub D6

(a) a purified or isolated nucleic acid encoding a secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit having at least 80% amino acid identity with the polypeptide of SEQ ID N°20 or 22, or a sequence complementary thereto;

At page 18, second paragraph:

B⁵

Once the optimal radioligand binding conditions have been determined, a test ligand can be introduced in the assay medium to evaluate the level of displacement of the radioligand. The concentration of test ligand to be introduced in the assay medium usually varies from 0.1 nM to about 100 μ M. A preferred test ligand concentration of about 10 μ M is usually a starting point in a high throughput screening assay. Then, depending on the number of hits obtained, it may be lowered or increased.

At page 18, sixth paragraph:

B⁶

The interaction of the secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptide with the flashplates can be optimized by adjusting the concentration of the polypeptide and by introducing a reagent which will favor this interaction. When a standard NEN Ni chelate flashplate is used, the secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptide volume usually varies between 0.5 and 20 μ l for a concentration of secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptide of 0.6 pmol/ μ l. As the published maximum binding capacity of

At page 19, first paragraph:

B⁷

NEN p plates is about 6 pmol per well, the inventors consider that an optimal concentration of secreted $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptide is probably around 5 pmol per well at 8 μ l.

At page 19, second paragraph:

B⁸

With regard to the reagent favoring the interaction between the secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptide and the radioligand as well as the flashplates, the inventors believe that imidazole could also be efficiently used for that purpose when the secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptide is tagged with an amino acid sequence including 6 histidine residues. The inventors also believe that imidazole concentrations can substantially enhance binding of the radioligand to the secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 polypeptide. The optimal concentration of imidazole used to enhance radioligand binding varies depending on the concentration of secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptide used in the assay. For instance, when the volume of the $\alpha_2\delta$ -1 subunit polypeptide is about 10 μ l ($\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 polypeptide concentration of 0.6 pmol/ μ l), the optimal imidazole concentration can vary between 1 and 20 mM, with a concentration of about 10 mM being preferred. As mentioned previously, other compounds such as histidine as well as pH variations may be used to enhance radioligand binding.

At page 19, fourth paragraph:

B⁹

Once the optimal radioligand binding conditions have been determined, a test ligand can be introduced in the assay medium to evaluate the level of displacement of the radioligand. The concentration of test ligand to be introduced in the assay medium usually varies from 0.1 nM to about 100 μ M. A preferred test ligand concentration of about 10 μ M is usually a

At page 20, fifth paragraph:

B¹⁰ Sub D²¹

In a further preferred embodiment, the polypeptide comprises an amino acid sequence having at least 80%, preferably 90%, more preferably 95%, and most preferably 98% or 99% amino acid identity with the amino acid sequence of SEQ ID N°4, SEQ ID N°5, SEQ ID N°6, SEQ ID N°10, SEQ ID N°11, SEQ ID N°12, SEQ ID N°16, SEQ ID N°17 and SEQ ID N°18.

At page 29, fourth paragraph:

Example 8

B¹¹

Ni Flashplate assay of [³H]Leucine binding to secreted soluble human $\alpha_2\delta$ -2-6His

The procedure described in example 7 is repeated, except that [³H]gabapentin is replaced by 25 μ l (10.1 nM) of [³H]Leucine (41 Ci/mmol).

At page 30, first indentation under **Example 9**:

B¹²

25 μ l 10 mM HEPES pH7.4 or 25 μ l of the test compound at the appropriate concentration in HEPES

At page 31, third paragraph:

Sub D³⁵

B¹³

Example 11

Construction of a nucleotide sequence encoding a soluble secreted mouse $\alpha_2\delta$ -3 deletion mutant of SEQ ID N°25 as follows.

a) Primer design

PCR primers were designed to generate the secreted soluble mouse $\alpha_2\delta$ -3 deletion mutant of SEQ ID N°24 as follows: